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# **Dermatopharmacokinetics: factors influencing drug clearance from the stratum corneum**

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## ABSTRACT

**Purpose.** The dermatopharmacokinetic methodology, in which tape stripping of the stratum corneum (SC) is used to access the amount of drug accumulated in the skin barrier, has been proposed for the quantification of topical drug bioavailability. This investigation examined the clearance phase of a model drug from the SC after a short application of an infinite dose.

**Methods.** A saturated solution of ibuprofen in propylene glycol/water was applied to the forearm of human volunteers for 30 minutes. The formulation was then removed and the drug profile across the SC was assessed immediately, and over the next 4 hours.

**Results.** The clearance phase depends only on drug diffusivity in the SC. However, the expected, progressive “flattening” of the concentration profiles with increasing time post-formulation removal was not observed. It was subsequently deduced, using infrared spectroscopy, that the rapid percutaneous diffusion of propylene glycol, relative to ibuprofen, resulted in the transient maintenance of a saturated drug concentration at the SC surface even after removal of the original formulation.

**Conclusions.** The important role of formulation excipients in topical delivery is demonstrated, and the local disposition of cosolvents within the SC may impact significantly on drug dermatopharmacokinetics and local bioavailability.

## Introduction

Optimization of topical drug bioavailability is an essential objective for the effective treatment of dermatological diseases. The assessment of the rate and the extent to which an active molecule attains its site of action on or within the skin, however, has not proved to be a facile task. Nevertheless, because of pressure to facilitate the entry of generic products into the market, regulatory agencies, such as the U.S. Food and Drug Administration (FDA), have been exploring alternative approaches with which to characterise a drug's dermatopharmacokinetics (DPK). One such method, involving the quantification of drug in the skin's barrier layer, the stratum corneum (SC), as a function of time post-application and post-removal of the formulation, involves sampling of the "biophase" by repeated adhesive tape-stripping. Since the release, and the subsequent withdrawal, of a draft guidance from the FDA proposing a specific protocol (1), a number of detailed studies into the potential usefulness and limitations of the methodology have been published (2-19).

The essence of a viable technique for evaluation of a drug's DPK (and for the subsequent determination of bioequivalence between different products) involves assessment of classic metrics such as the maximum quantity of the active in the SC ( $A_{\max}$ ), the time at which that maximum level is reached ( $T_{\max}$ ), and the area under the SC level versus time profile (AUC). Unlike the situation with oral drug administration,  $A_{\max}$ ,  $T_{\max}$  and AUC in the SC are not only dependent on the kinetics of absorption, they are also influenced importantly by the time at which drug administration is stopped by removal of any remaining formulation on the skin surface. Because percutaneous absorption is often a slow process, it is quite conceivable, and often the case, that the applied product will be removed (either deliberately or inadvertently) before the absorption phase is completed.

The majority of investigations undertaken to-date has focussed upon the absorption phase of DPK (2, 4, 8, 10, 11). These experiments have demonstrated the importance of quantifying the extent to which the SC is removed during tape-stripping, as well as providing evidence that the method can sensitively distinguish between formulations that are not equivalent (11). It has also been possible to interpret drug concentration profiles across the SC so as to deduce partitioning and diffusion parameters, which characterize the absorption process and which can subsequently be used to predict an entire absorption profile from a single short-contact duration experiment (10).

Less attention, though, has been given to the elimination, or clearance, phase of the DPK profile, which, as articulated above, may have a significant impact on the key metrics classically derived from bioavailability and bioequivalence studies. Because topical formulations are typically complex, involving excipients which are volatile, or which can solubilize the drug within the outer SC but penetrate at different rates, or which act as enhancers that increase the permeability of the skin, there are manifold ways in which the constituents of a vehicle may influence DPK. The present study addresses, therefore, the clearance of a model drug, ibuprofen, subsequent to its application and removal in a DPK experiment. Ibuprofen, applied as a saturated solution in a 75:25 propylene glycol/water mixture, has been chosen for this work because a detailed examination of its absorption kinetics has already been undertaken and reported (9-11). Attention is focussed not only on the disposition of the active agent, but also on that of the common cosolvent employed, namely propylene glycol.

## **Materials and Methods**

### **1. Chemicals**

Racemic ibuprofen and propylene glycol were purchased from Sigma (Dorset, UK). Acetonitrile (HPLC grade) was obtained from Fisher Scientific (Loughborough, UK). All other chemicals were of analytical grade.

### **2. Ibuprofen solubility and HPLC assay**

The solubility of ibuprofen at 20°C in 75:25 (v/v) propylene glycol/water was determined as previously described (9) and was found to be  $9.6 \pm 0.7$  mg/ml.

The method was modified from that reported previously (9). The HPLC apparatus from Dionex (Surrey, UK) comprised: SOR-100 solvent rack, P680 pump, ASI-100 automated sample injector, PD40 UV-Vis detector; Chromeleon software; C18 Acclaim 120 column (4.6 x 250 mm; 5  $\mu$ m). The eluent was a 55:45 mixture of acetonitrile and 0.1 M citrate buffer at pH 2.4, pumped at a rate of 1.5 ml/min. Ibuprofen was detected at 223 nm with a retention time of about 5 minutes. Linearity was obtained in the range of 0.5-20  $\mu$ g/ml; reproducibility, calculated as %RSD was 0.8%. No interfering peaks from either the tape adhesive or the SC were apparent.

### **3. In vivo DPK experiments - Ibuprofen**

Five volunteers (3 female, 2 male, aged 24-60 years) with no history of dermatological disease participated in the study, which was approved by the local research ethics committee (Royal United Hospital, Bath, UK). Informed consent was obtained from each subject. The procedures followed have been reported in the literature (2) and involved two distinct components.

In a first set of experiments, SC thickness for each volunteer was determined at a site on the ventral forearm adjacent to those used subsequently for the DPK

measurement. A circular template (diameter: 2 cm) delimited a specific skin area at which a baseline measurement of transepidermal water loss (TEWL) was made in triplicate (Aquaflux, Biox Systems Ltd, London, UK) and averaged. Pre-weighed adhesive tapes (2.5 X 2.5 cm, Scotch Booktape #845, 3M Co., St Paul, MN) were then sequentially applied over the template-delimited area, rubbed back and forth six times with stainless steel tweezers, and then removed to collect progressively layers of the SC. Between each strip, TEWL was measured as before. Stripping continued until the value of TEWL had reached 4 times the baseline or 60-70 g/m<sup>2</sup>/h, which corresponds to the removal of at least 75% of the total SC thickness (20) Each tape was reweighed to determine the mass of SC removed. Then, knowing the area of the SC stripped, and assuming a SC density equal to 1 g/cm<sup>3</sup> (21), the thickness of the SC removed on each strip and the cumulative thickness removed, were calculated. Finally, following a published procedure (20), values of 1/TEWL were plotted against the cumulative SC thickness removed (μm), yielding a straight line, the x-axis intercept of which equalled the SC thickness. This exercise permitted all subsequent DPK data to be normalized for each subject and presented as concentration profiles as a function of relative SC depth (from 0 at the surface to a value of 1 at the inner boundary of the SC).

Secondly, ibuprofen DPK measurements were then conducted on the ventral forearms of the same volunteers. Drug application involved a saturated solution (~10 mg/ml) in 75:25 (v/v) propylene glycol/water. The solution applied (500 μl) was constrained by a plastic template (diameter: 2.5 cm) to prevent spreading and spillage, and was covered with an occlusive bandage during the 30-minute administration period. At the end of the treatment, excess solution was removed, and the SC surface was blotted completely dry with absorbent tissue. The site was then either stripped immediately or left unoccluded for 30 minutes or 1,2,3 or 4 hours before stripping. In one further set of experiments, a delay time of 30 minutes was again employed but the treated site was occluded during this

period (Figure 1). The tape-stripping and TEWL measurement procedures described previously (for the SC thickness determination) were again adopted here, but with less frequent determinations of TEWL to allow the strips to be collected as quickly as possible.

Once tape-stripping had concluded, the tapes were reweighed and then placed individually in glass vials containing 1 ml of a 75:25 (v/v) mixture of acetonitrile and water into which ibuprofen was extracted over the next 4 hours (8). Recoveries were >99%. Finally, the extracts were assayed by HPLC for ibuprofen using the method described above. Results were then expressed as ibuprofen concentration profiles in the SC ( $\mu\text{mol}$  of drug per mg of SC) as a function of the relative depth into the barrier ( $x/L$  where  $x$  is the cumulative depth and  $L$  the absolute thickness of the SC in  $\mu\text{m}$ )

#### **4. In vivo DPK experiments - Propylene glycol**

A separate set of experiments was undertaken in the same volunteers to track the disposition of the cosolvent, propylene glycol. In this case, detection of the chemical utilized attenuated total reflectance, Fourier-transform infrared spectroscopy (ATR-FTIR) (22). The spectrophotometer (Nicolet 520P, Madison, WI) was equipped with a mercury–cadmium–telluride detector and a ZnSe reflectance element ( $45^\circ$ ,  $7 \times 1 \times 0.2$  cm, Spectra-Tech, Inc., Shelton, CT). Spectra (32 scans,  $4 \text{ cm}^{-1}$  resolution) were collected over the range of  $4000$  to  $400 \text{ cm}^{-1}$ , with particular attention focussed on the C-O stretching vibration from propylene glycol at  $1040 \text{ cm}^{-1}$ .

A large template ( $8 \times 2 \text{ cm}^2$ ) was used to provide a treated skin area comparable to that of the ATR reflectance element. Application of a 75:25 (v/v) mixture of propylene glycol and water lasted 30 minutes, at the end of which excess liquid was removed and the skin surface completely cleaned as before. The deposition of propylene glycol in the SC was then evaluated immediately or after a 30-minute delay, during which time the treated area was either kept under occlusion or left open to ambient conditions. The fate of



the cosolvent was monitored in two different ways: either at skin surface at a function of time, or within the SC via the use of tape-stripping.

In the former case, post cleaning of the skin, the volunteer's arm was positioned and remained on the ATR reflectance element for the next 30 minutes. Spectra were recorded every 5 minutes, which was considered a reasonable period with respect to the time necessary for the spectrum to be recorded (~0.5 minutes) and sufficient to allow for a measurable amount of co-solvent transport to have occurred; in this first instance, therefore, the application site was effectively occluded during the data collection period. A subsequent experiment was then performed in which the arm was removed from the reflectance element between each spectrum acquisition; in other words, under non-occluded conditions. Because the degree of skin contact with the ZnSe crystal is not reproducible, data from these measurements are presented as the semi-quantitative ratio of the area under the C-O absorbance peak at  $1040\text{ cm}^{-1}$  from propylene glycol to that under the amide I and amide II bands (originating from carbonyl stretching and N-H bending vibrations from SC keratin) (23-26) (Figure 2).

In the latter case, the propylene glycol profiles across the SC were determined. First, after the 30-minute treatment and post-cleaning, the skin was immediately tape-stripped. Each strip was rapidly transferred to the ZnSe crystal for an IR spectrum to be recorded. While this was being carried out, the next tape had been applied and was able thereby to keep the treated site occluded. Due to the volatility of propylene glycol, and the risk that the cosolvent might be inadvertently "lost" (27), all transfers were performed as quickly as possible and the tapes, in this series of experiments, were not weighed before and after stripping as they has been in the ibuprofen component of the work. The procedure, as outlined, was then conducted on two further occasions after a 30-minute delay post-application of the vehicle and following surface cleaning. In one instance, the treated site was occluded during the delay; in the other, it was not.

For these experiments, in which propylene glycol absorbance on the tapes as a function of the depth into the SC was determined, it was possible to develop a calibration curve so that the results could be expressed quantitatively in terms of the amount of cosolvent per  $\text{cm}^2$  of SC. This was achieved with a method previously reported (28) wherein tape-strips, on which removed SC was present, were “doped” with different amounts of propylene glycol and then subsequently read by ATR-FTIR. The calibration curve covered the range of 7 to 70  $\mu\text{g}/\text{cm}^2$  over which linearity was excellent. Above 13  $\mu\text{g}/\text{cm}^2$ , the percentage relative error and the relative standard deviation were below 20%. Table I summarises the details of this analytical validation.

## Results and Discussion

The first DPK experiment involved determination of the ibuprofen concentration profile across the SC immediately following a 30-minute application of the drug as a saturated solution in 75:25 (v/v) propylene glycol/water. The results from the five volunteers are in Figure 3 and are presented as a function of relative depth into the barrier, a normalization procedure made possible by the independent assessment of individual SC thicknesses as described above. Despite the relatively small number of subjects involved in this study, the data were reasonably reproducible and in line with recently published work (10). As before, the profiles were fitted to the applicable solution to Fick's second law of diffusion (7), assuming that drug diffusivity in the SC is slow compared to uptake by the cutaneous microcirculation, and that "sink" conditions apply for the drug at the SC-viable epidermis interface:

$$C_x = KC_v \left[ 1 - \frac{x}{L} - \frac{2}{\pi} \sum_{m=1}^{\infty} \frac{\exp\left[-m^2 \pi^2 t_{app} \left(\frac{D}{L^2}\right)\right] \sin(m \pi x/L)}{m} \right] \quad \text{Eq. 1}$$

where  $C_x$  is the drug concentration at position  $x$  in the SC at time  $t_{app}$  and  $C_v$  is the drug concentration in the vehicle. The fitting yields values for the drug's SC-vehicle partition coefficient ( $K$ ) and for  $D/L^2$ , a first order rate constant comprising the ratio of the drug diffusivity ( $D$ ) in the SC to the thickness ( $L$ ) squared of the barrier (2). The derived results for  $K$  and  $D/L^2$  are also given in Figure 3; the means ( $\pm$  SD) were 2.99 ( $\pm 0.66$ ) and 0.21 ( $\pm 0.04$ )  $\text{h}^{-1}$ , respectively, and were not significantly different from those reported previously (7). The integrated amounts in the SC were derived from the areas under the profiles in Figure 3 and yielded a mean ( $\pm$  SD) value of 12.2 ( $\pm 3.5$ )  $\mu\text{g}/\text{cm}^2$ . The coefficient of variation of the DPK parameters obtained was around 20% supporting the robust nature of this *in vivo* experiment and the analysis of the resulting data.

The next phase of investigation was to evaluate the clearance of drug from the SC as a function of time post-removal of the applied formulation. While uptake in the SC is controlled by partitioning and diffusion, clearance is dominated by diffusion alone. In this case, assuming that the drug is non-volatile (i.e., that it is not lost from the SC to the air by evaporation), the concentration profile during the clearance phase is described by Eq. 2 (29) (see Appendix):

$$C_x = 2KC_v \sum_{n=0}^{\infty} \left\{ \left[ \frac{1}{\lambda_n^2} - 2 \sum_{m=1}^{\infty} \frac{\exp[-m^2 \pi^2 t_{app} (D/L^2)]}{m^2 \pi^2 - \lambda_n^2} \right] \cos(\lambda_n x / L) \exp[-\lambda_n^2 (t - t_{app}) (D/L^2)] \right\} \quad \text{Eq. 2}$$

where  $C_x$  is again the drug concentration at position  $x$  in the SC,  $t_{app}$  is the time for which the formulation was applied and  $\lambda_n = (2n+1)\pi/2$ . Using the average values of  $K$  and  $D/L^2$  from the initial DPK measurements and setting  $(t - t_{app})$  equal to 0.5, 1, 2, 3 and 4 hours following an application time of 30 minutes, Equation 2 was used to generate predicted ibuprofen concentration profiles across the SC during the clearance phase. These results are compared with the experimental data subsequently obtained in Figure 4. The absence of agreement between theory and experiment is clear. The anticipated decay and flattening of the concentration profile is not observed; in fact, the total amount of ibuprofen (in  $\mu\text{g}/\text{cm}^2$ ) within the SC hardly changed at all during the 4 hours clearance period (Table II).

The DPK literature on ibuprofen delivery into the SC reveals that uptake from saturated solutions containing different percentages of propylene glycol was far from constant, with significantly more drug being made available as the presence of the cosolvent increased (9). The simplest explanation for these findings is that propylene glycol itself entered in the SC in significant amount and thereby enabled the solubility of the drug in the membrane to be increased. Once this has been achieved and the driving force for diffusion removed (i.e., upon removal of the formulation to initiate the clearance

phase), the elimination of both drug and cosolvent from the SC will proceed. However, if propylene glycol clearance is faster than that of ibuprofen (a possible scenario given the difference in their molecular weights and recognising that propylene glycol is somewhat volatile), the enhanced solubility of the drug in the SC will not be sustained, it will precipitate within the SC and, hence, be unable to diffuse out of the membrane. Subsequent experiments examined this hypothesis.

First, the ibuprofen profile across the SC was measured once again after a 30-minute delay between the 30-minute application and the tape-stripping procedure. However, in this case, the treated site was occluded during the delay period, rather than being left open to ambient conditions as before (Figure 4). The impact of this modification is apparent in Figure 5, which shows that, when propylene glycol was prevented from evaporative loss at the SC surface, ibuprofen remained able to diffuse from the SC. While the concentration profile does not overlay perfectly the predicted behaviour from Eq. 2, the change relative to the unoccluded experiments is definitely in the right direction.

Second, rather than infer the behaviour of propylene glycol at the skin surface, ATR-FTIR was performed to track the presence of the cosolvent following termination of the 30-minute application period. The characteristic absorbance from propylene glycol C-O stretching was used to assess the disappearance of the compound over a period of one hour from the outermost layer of the SC, either when the site was occluded or unoccluded. Figure 6 demonstrates that the cosolvent was lost by both evaporation and diffusion into and through the SC (as has been previously observed in a detailed investigation of minoxidil delivery from vehicles which also contained ethanol (27)), in that more propylene glycol disappeared from the surface when it was not protected between IR measurements. Roughly speaking, over the 1-hour measurement period, about 30% of the initial signal was lost when the site was occluded, compared to nearly 60% when it was not, suggesting

that evaporative and diffusive clearance mechanisms contributed about equally to the results obtained.

Third, a further DPK study was undertaken to evaluate the concentration profile of propylene glycol itself across the SC under different experimental conditions. Skin was treated with 75:25 (v/v) propylene glycol/water for 30 minutes, cleaned and then either (i) stripped immediately, or (ii) either occluded for a further 30 minutes, or left open to ambient conditions for 30 minutes, and then stripped. Propylene glycol in the tape-strips was quantified by ATR-FTIR using a previously validated calibration procedure described in the Materials & Methods. Immediately post-application there was a steep concentration gradient of the cosolvent across the SC (Figure 7). The area under the profile provides the cumulative amount of propylene glycol in the SC at this time:  $150 \pm 27 \mu\text{g}/\text{cm}^2$ , a value in good agreement with a report in the literature describing the quantification of the compound by GC-MS in human skin following a 30-minute application of the pure material ( $110 \pm 40 \mu\text{g}/\text{cm}^2$ ) (30). When the treated skin was tape-stripped after a 30-minute delay, during which time the treated area was kept under occlusion, the propylene glycol concentration profile decreased (Figure 7) and the amount recovered from the SC fell to  $59 (\pm 16) \mu\text{g}/\text{cm}^2$ . Repeating the experiment with the treated site unoccluded during the 30-minute delay both reduced the amount of cosolvent recovered ( $84 (\pm 10) \mu\text{g}/\text{cm}^2$ ) and altered the shape of the profile (Figure 7). Notably, there was significantly less propylene glycol in the surface SC layers, presumably because the compound had now been lost from the barrier both by diffusion through the membrane and by evaporation to the atmosphere (although which of these two loss processes dominates is difficult to deduce (27)).

## **Conclusions**

In summary, the results reported in this paper confirm that the disposition and fate of a topically applied drug are not only dependent upon its physicochemical properties (size, lipophilicity, etc.), but also upon the manner in which the active species is presented to the skin; that is, the formulation. Even with the simple cosolvent vehicle employed here, the behaviour of one constituent (propylene glycol) is also complex and has a direct influence on the drug's dermatopharmacokinetics, not only during the uptake phase, as has already been examined in depth in other work, but also during the clearance of the active from the SC. It follows that comparison between putatively bioequivalent topical drug products must involve careful examination of the potential effects of different excipients and the manner in which their behaviour may impact on the rate and extent at which the active attains its site of action. It should also be emphasised that the behaviour observed in this research may be even more pronounced when the amount of co-solvent applied is similar to that typically used in clinically-approved products (as opposed to the higher levels which have been used here).

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## Appendix

The concentration of drug in the stratum corneum (SC) during clearance is described by Fick's second law:

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \quad \text{Eq. A1}$$

where the concentration at time  $t_{\text{app}}$  is defined by Eq. 1, the flux of drug from the surface of the stratum corneum is zero, and sink conditions apply at the interface between the SC and viable epidermis:

$$\frac{\partial C}{\partial x} = 0 \quad \text{at } x = 0$$

$$C = 0 \quad \text{at } x = L$$

Eq. A1 is solved for these boundary conditions by separation of variables to obtain Eq. 2 (19).



## References:

1. HHS, FDA, and CDER. Topical dermatological drug product NDAs and ANDAs – in vivo bioavailability, bioequivalence, in vitro release and associated studies *Draft Guidance for Industry* (1998).
2. Y. N. Kalia, I. Alberti, A. Naik, and R. H. Guy. Assessment of topical bioavailability in vivo: the importance of stratum corneum thickness. *Skin Pharmacol Appl Skin Physiol* **14 Suppl 1**: 82-6 (2001).
3. L. K. Pershing, J. L. Nelson, J. L. Corlett, S. P. Shrivastava, D. B. Hare, and V. P. Shah. Assessment of dermatopharmacokinetic approach in the bioequivalence determination of topical tretinoin gel products. *J Am Acad Dermatol* **48**: 740-51 (2003).
4. M. B. Reddy, A. L. Stinchcomb, R. H. Guy, and A. L. Bunge. Determining dermal absorption parameters in vivo from tape strip data. *Pharm Res* **19**: 292-8 (2002).
5. V. P. Shah. IV-IVC for topically applied preparations--a critical evaluation. *Eur J Pharm Biopharm* **60**: 309-14 (2005).
6. E. Benfeldt, S. H. Hansen, A. Volund, T. Menne, and V. P. Shah. Bioequivalence of topical formulations in humans: evaluation by dermal microdialysis sampling and the dermatopharmacokinetic method. *J Invest Dermatol* **127**: 170-8 (2007).
7. M. Breternitz, M. Flach, J. Prassler, P. Elsner, and J. W. Fluhr. Acute barrier disruption by adhesive tapes is influenced by pressure, time and anatomical location: integrity and cohesion assessed by sequential tape stripping. A randomized, controlled study. *Br J Dermatol* **156**: 231-40 (2007).
8. C. Herkenne, A. Naik, Y. N. Kalia, J. Hadgraft, and R. H. Guy. Pig ear skin ex vivo as a model for in vivo dermatopharmacokinetic studies in man. *Pharm Res* **23**: 1850-6 (2006).
9. C. Herkenne, A. Naik, Y. N. Kalia, J. Hadgraft, and R. H. Guy. Effect of propylene glycol on ibuprofen absorption into human skin in vivo. *J Pharm Sci* (2007).
10. C. Herkenne, A. Naik, Y. N. Kalia, J. Hadgraft, and R. H. Guy. Dermatopharmacokinetic prediction of topical drug bioavailability in vivo. *J Invest Dermatol* **127**: 887-94 (2007).
11. C. Herkenne, A. Naik, Y. N. Kalia, J. Hadgraft, and R. H. Guy. Ibuprofen transport into and through skin from topical formulations: in vitro-in vivo comparison. *J Invest Dermatol* **127**: 135-42 (2007).
12. I. Jakasa, M. M. Verberk, M. Esposito, J. D. Bos, and S. Kezic. Altered penetration of polyethylene glycols into uninvolved skin of atopic dermatitis patients. *J Invest Dermatol* **127**: 129-34 (2007).
13. J. Lademann, A. Ilgevicus, O. Zurbau, H. D. Liess, S. Schanzer, H. J. Weigmann, C. Antoniou, R. V. Pelchrzim, and W. Sterry. Penetration studies of topically applied substances: Optical determination of the amount of stratum corneum removed by tape stripping. *J Biomed Opt* **11**: 054026 (2006).
14. B. N'Dri-Stempffer, W. C. Navidi, R. H. Guy, and A. L. Bunge. Improved Bioequivalence Assessment of Topical Dermatological Drug Products Using Dermatopharmacokinetics. *Pharm Res* (2008).
15. B. N'Dri-Stempffer, W. C. Navidi, R. H. Guy, and A. L. Bunge. Optimizing metrics for the assessment of bioequivalence between topical drug products. *Pharm Res* **25**: 1621-30 (2008).
16. C. Pellanda, E. Ottiker, C. Strub, V. Figueiredo, T. Rufli, G. Imanidis, and C. Surber. Topical bioavailability of triamcinolone acetonide: effect of dose and application frequency. *Arch Dermatol Res* **298**: 221-30 (2006).

17. C. Pellanda, C. Strub, V. Figueiredo, T. Rufli, G. Imanidis, and C. Surber. Topical bioavailability of triamcinolone acetonide: effect of occlusion. *Skin Pharmacol Physiol* **20**: 50-6 (2007).
18. S. Wiedersberg, C. S. Leopold, and R. H. Guy. Dermatopharmacokinetics of betamethasone 17-valerate: Influence of formulation viscosity and skin surface cleaning procedure. *Eur J Pharm Biopharm* (2008).
19. S. Wiedersberg, A. Naik, C. S. Leopold, and R. H. Guy. Pharmacodynamics and dermatopharmacokinetics of betamethasone 17-valerate: assessment of topical bioavailability. *Br J Dermatol* (2008).
20. Y. N. Kalia, I. Alberti, N. Sekkat, C. Curdy, A. Naik, and R. H. Guy. Normalization of stratum corneum barrier function and transepidermal water loss in vivo. *Pharm Res* **17**: 1148-50 (2000).
21. R. L. Anderson and J. M. Cassidy. Variation in physical dimensions and chemical composition of human stratum corneum. *J Invest Dermatol* **61**: 30-2 (1973).
22. N. Higo, A. Naik, D. B. Bommannan, R. O. Potts, and R. H. Guy. Validation of reflectance infrared spectroscopy as a quantitative method to measure percutaneous absorption in vivo. *Pharm Res* **10**: 1500-6 (1993).
23. I. Alberti, Y. N. Kalia, A. Naik, J. Bonny, and R. H. Guy. Effect of ethanol and isopropyl myristate on the availability of topical terbinafine in human stratum corneum, in vivo. *Int J Pharm* **219**: 11-9 (2001).
24. I. Alberti, Y. N. Kalia, A. Naik, J. D. Bonny, and R. H. Guy. In vivo assessment of enhanced topical delivery of terbinafine to human stratum corneum. *J Control Release* **71**: 319-27 (2001).
25. V. H. Mak, R. O. Potts, and R. H. Guy. Percutaneous penetration enhancement in vivo measured by attenuated total reflectance infrared spectroscopy. *Pharm Res* **7**: 835-41 (1990).
26. M. Pellett, A. Watkinson, J. Hadgraft, and K. Brain. Comparison of permeability data from traditional diffusion cells and ATR-FTIR spectroscopy. Part II: determination of diffusional pathlengths in synthetic membranes and human stratum corneum. *Int. J. Pharm* **154**: 217-227 (1997).
27. J. C. Tsai, M. J. Cappel, G. L. Flynn, N. D. Weiner, J. Kreuter, and J. J. Ferry. Drug and vehicle deposition from topical applications: use of in vitro mass balance technique with minoxidil solutions. *J Pharm Sci* **81**: 736-43 (1992).
28. A. L. Stinchcomb, F. Pirot, G. D. Touraille, A. L. Bunge, and R. H. Guy. Chemical uptake into human stratum corneum in vivo from volatile and non-volatile solvents. *Pharm Res* **16**: 1288-93 (1999).
29. H. S. Carslaw and J. C. Jaeger. *Conduction of Heat in Solids* Oxford University Press, Oxford, UK, 1959, pp. 92-110.
30. I. Schneider, B. Dobner, R. Neubert, and W. Wohlrab. Evaluation of drug penetration into human skin ex vivo using branched fatty acids and propylene glycol. *Int J Pharm* **145**: 187-196 (1996).

**Table I: Details of the analytical method used to quantify propylene glycol on tape-strips of SC. The linear calibration curve was  $y = 4.87 + 0.69x$ ;  $r^2 = 0.93$**

Nominal amount of propylene glycol ( $\mu\text{g}/\text{cm}^2$ )	Fitted value ( $\mu\text{g}/\text{cm}^2$ )	n	RSD%	ER %
6.9	11.1	7	21.7	60.1
13.8	12.2	7	16.95	13.6
27.6	23.2	7	19.22	16.79
35.7	38.8	5	17.79	17.65
57.1	56.1	5	6.30	4.88
71.4	81.9	1	-	14.7

**Table II: Quantity of ibuprofen (mean  $\pm$  SD, n=5) in the SC following a 30-minute application of a saturated drug solution in 75:25 (v/v) propylene glycol/water under different clearance conditions as specified in the text.**

Delay (h)	Ibuprofen ( $\mu\text{g}/\text{cm}^2$ )
0	$13.6 \pm 4.1$
0.5	$17.4 \pm 0.9$
1	$10.2 \pm 1.8$
2	$9.3 \pm 1.4$
3	$10.5 \pm 4.6$
4	$9.7 \pm 2.9$
0.5 (occluded)	$7.3 \pm 1.0^a$

<sup>a</sup>Value statistically different from those when the delay was either 0 or 0.5 h ( $p < 0.05$ ). The significance of any differences between values was assessed using ANOVA followed by the Bonferroni test.

## Figure legends

- Figure 1.** Schematic representation of the DPK experiments performed to assess the clearance of ibuprofen from the SC following its topical application to the skin.
- Figure 2.** Typical ATR-FTIR spectrum from human skin *in vivo* after a 30-minute contact with a 75:25 (v/v) mixture of propylene glycol and water. The C-O stretching absorbance band from the cosolvent and the amide I and amide II absorbance from SC keratin are highlighted.
- Figure 3.** Ibuprofen concentration profiles across the SC of 5 volunteers immediately following a 30-minute application of a saturated drug solution in 75:25 (v/v) propylene glycol/water. The best fits of Eq. 1 to the data are represented by the solid lines, and the derived value (mean  $\pm$  SD) of the drug's SC/vehicle partition coefficient (K) and diffusivity parameter ( $D/L^2$ ), as well as the total uptake of drug into the SC (AUC) are shown in the table insert.
- Figure 4.** (A) Theoretically predicted concentration profiles of ibuprofen across the SC following a 30-minute treatment, followed by various periods of delay during which the drug is expected to clear from the skin. The profiles were determined using Eq. 2 and the average values of K and  $D/L^2$  derived from the data in Figure 3. (B) Example of experimentally determined results following the protocol outlined in Figure 1(a).
- Figure 5.** Typical ibuprofen concentration profiles across the SC following a 30-minute application of a saturated solution in 75:25 (v/v) propylene glycol/water and then either (i) tape-stripping Immediately (filled circles), or (ii) tape-stripped after a further 30-minutes during which time the skin was either occluded (filled squares) or open to ambient conditions (open circles). The dotted line represents theoretical "delay = 0" curve calculated using Eq.1; the solid line represents the theoretical "delay = 30 minutes" curve calculated using Eq.2.
- Figure 6:** Relative levels (determined by ATR-FTIR) of propylene glycol remaining in the SC as a function of time after a 30-minute application of a 75:25 (v/v) mixture of cosolvent and water. Post treatment and cleaning of the skin surface, the site was either maintained under occlusion (closed symbols) or left open to the ambient condition in between the acquisition of spectra (open

symbols). The data have been corrected by the background signal at 1040  $\text{cm}^{-1}$  observed when the SC has not been exposed to propylene glycol.

**Figure 7:** Propylene glycol concentration profiles (mean  $\pm$  SD; n=5) across the SC (expressed in terms of tape strip number) following a 30-minute application of a 75:25 (v/v) mixture of cosolvent and water and then either (i) tape-stripping immediately (filled circles), or (ii) tape stripped after a further 30 minutes during which time the skin was either occluded (filled squares) or open to ambient conditions (open circles).

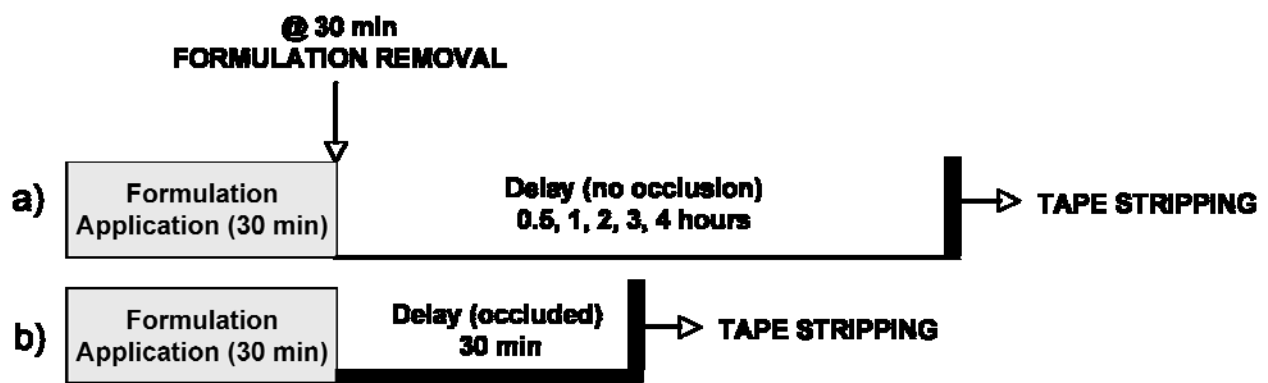


Figure 1

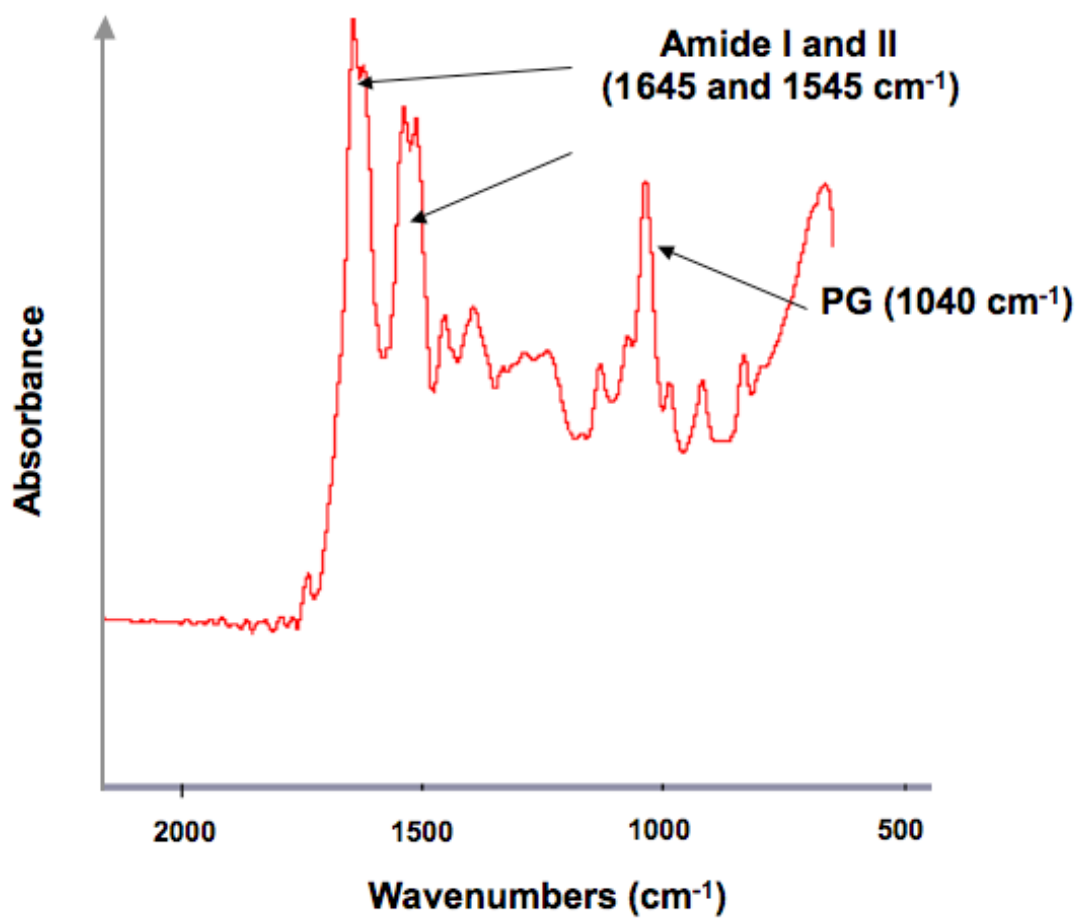


Figure 2



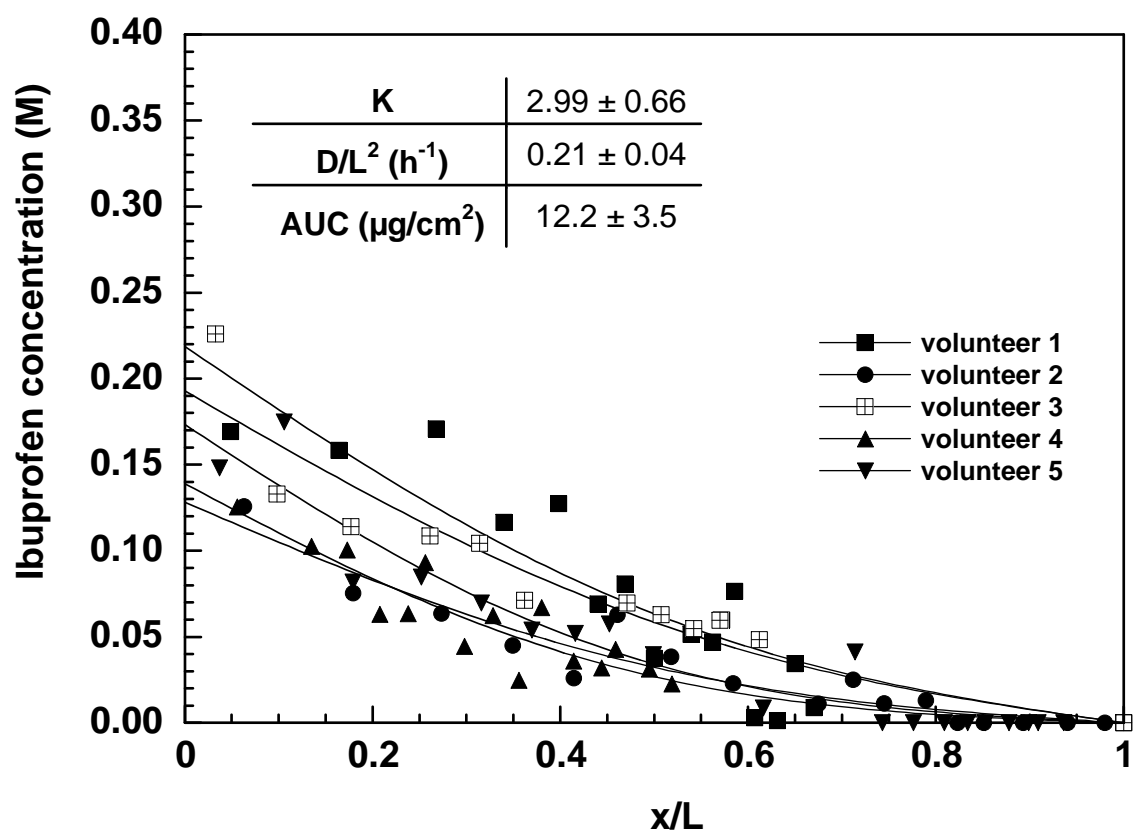


Figure 3.

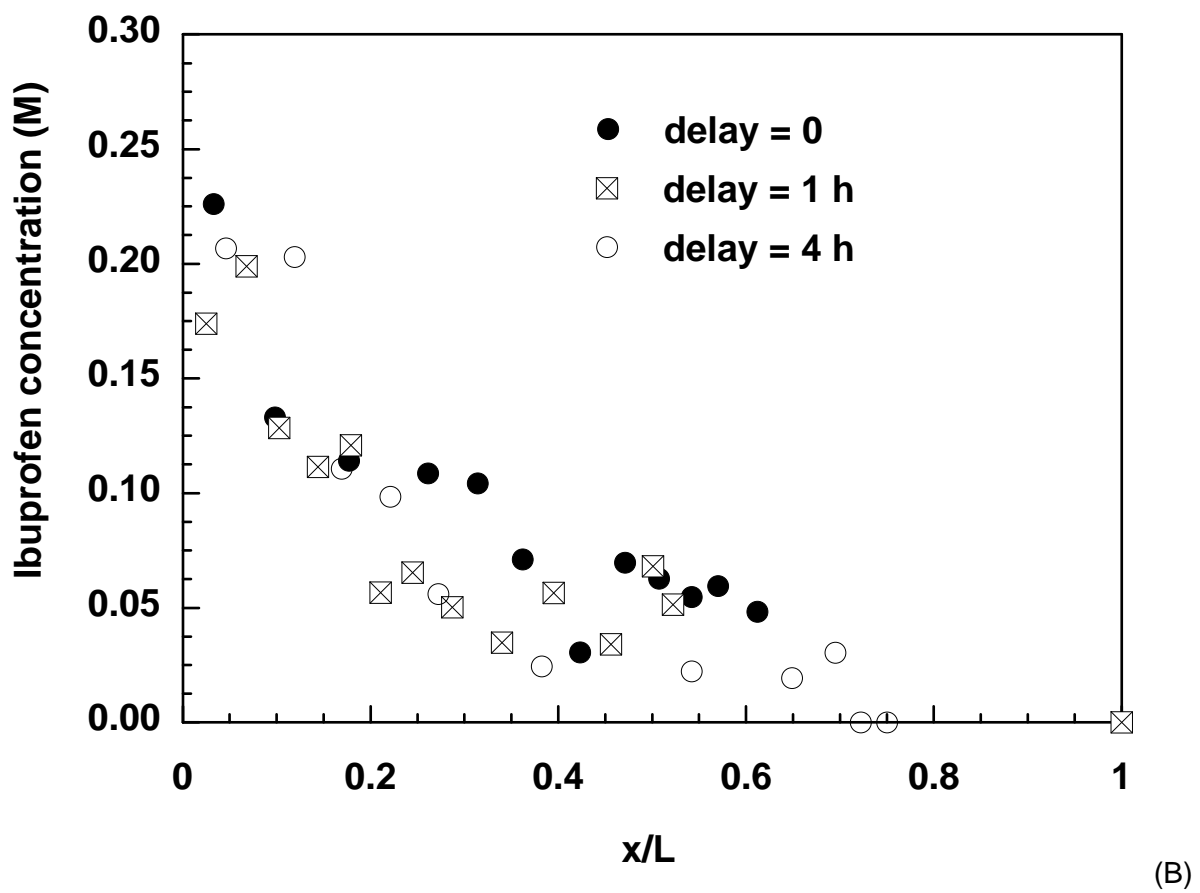
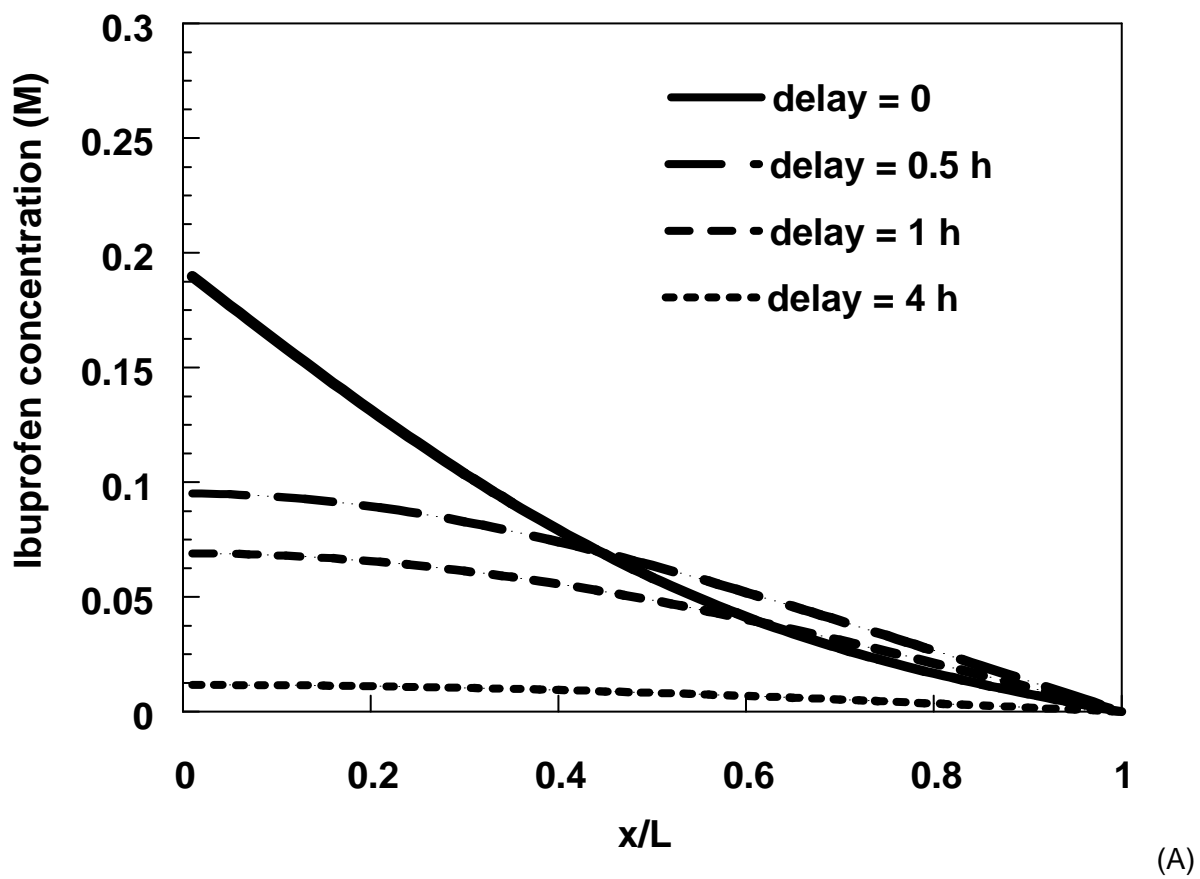


Figure 4.

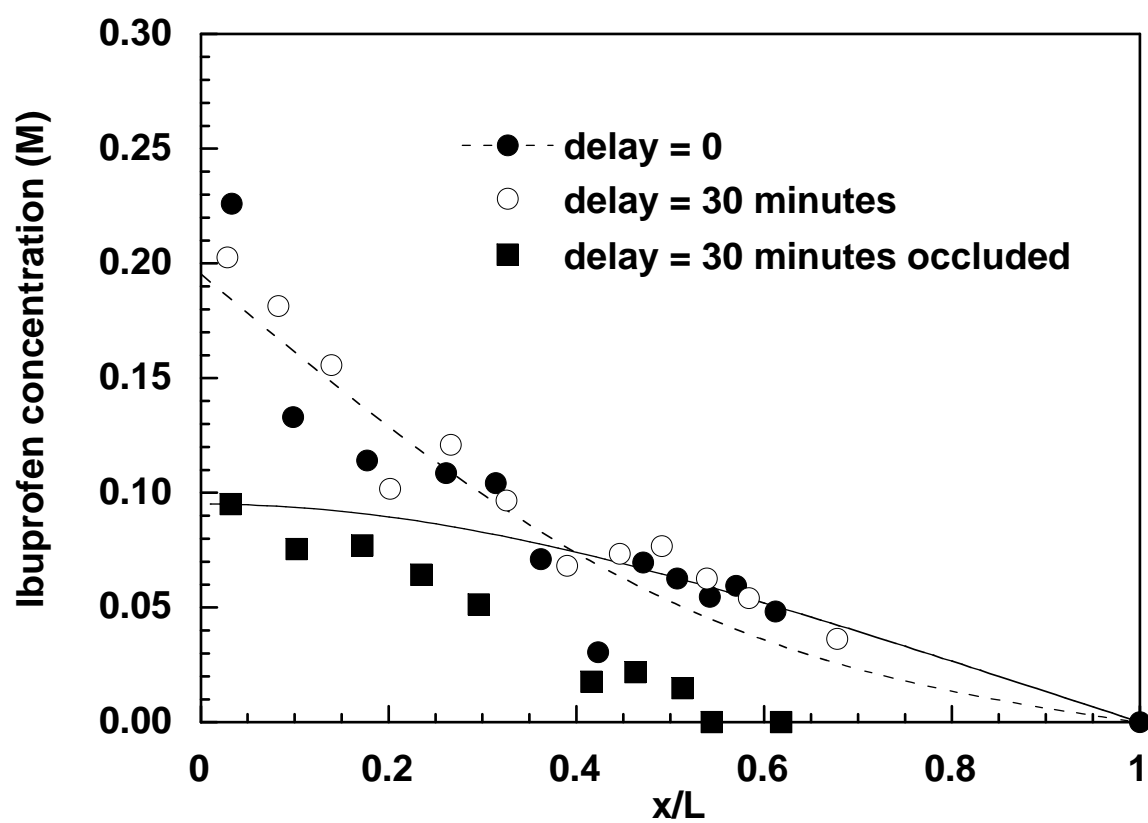


Figure 5.

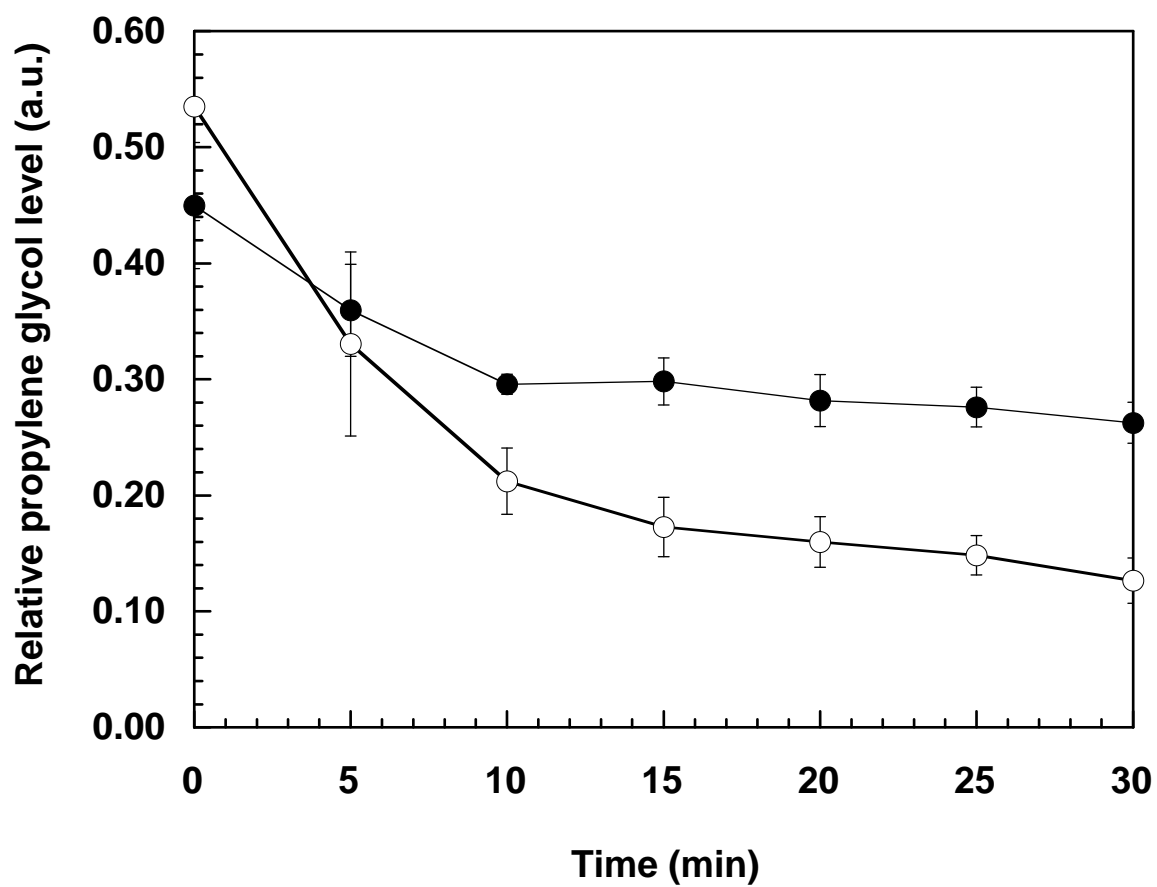


Figure 6

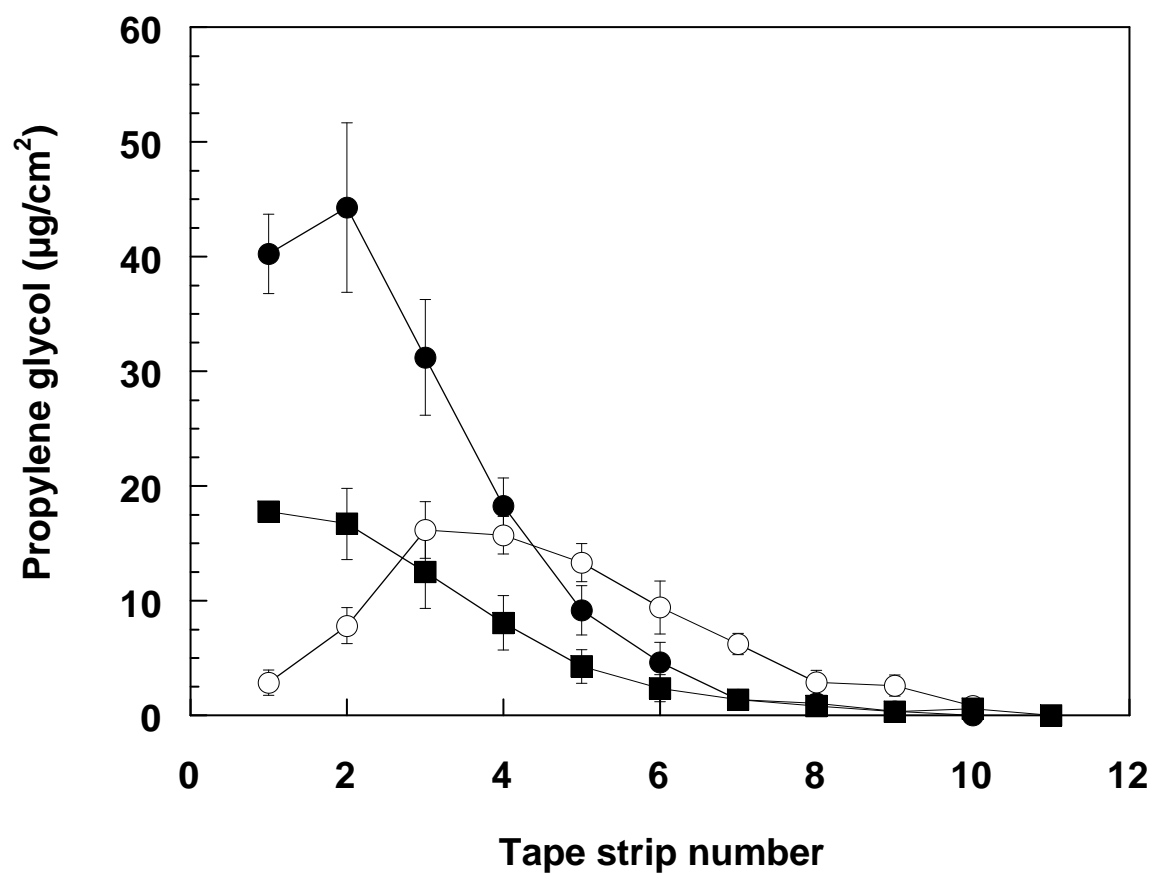


Figure 7